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EXAMINER	
NGUYEN, QUANG	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/672,484	CONTRERAS ET AL.	
	Examiner	Art Unit	
	QUANG NGUYEN, Ph.D.	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *13 June 2008*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *74-113* is/are pending in the application.

4a) Of the above claim(s) *74-89* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *90-113* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's election with traverse of the following species in the reply filed on 6/13/08 is acknowledged. Applicants elected: (a) fungal glucosidase II and (b) GAP promoter.

Upon further consideration and in light of the prior art rejections applied below, the species restriction requirement is withdrawn.

Claims 74-113 are pending in the present application.

This application contains claims 74-89 drawn to an invention nonelected with traverse in the reply filed on 8/9/06. A complete reply to the final rejection must include cancellation of nonelected claims or other appropriate action (37 CFR 1.144) See MPEP § 821.01.

Accordingly new claims 90-113 are examined on the merits herein.

Response to Amendment

The rejection under 35 U.S.C. 112, first paragraph, for the Lack of Written Description was withdrawn in light of Applicant's amendment.

The rejection under 35 U.S.C. 102(b) as being anticipated by Martinet et al. (Biotechnology Letters 20:1171-1177, 1998; IDS) as evidenced by the pPICZB vector diagram (Invitrogen Catalog, 1998; IDS) was withdrawn in light of Applicant's amendment.

The rejection under 35 U.S.C. 102(b) as being anticipated by Chiba et al. (J. Biol. Chem. 41:26298-26304, 1998; IDS) as evidenced by Inoue et al. (Biochim. Biophys. Acta 1253:141-145, 1995; IDS) was withdrawn in light of Applicant's amendment.

The rejection under 35 U.S.C. 102(b) as being anticipated by JP 8-336387 (IDS) was withdrawn in light of Applicant's amendment.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

New claims 107-113 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. ***This is a new ground of rejection necessitated by Applicant's amendment.***

New claims 107-113 are rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential elements, such omission amounting to a gap between the elements. See MPEP § 2172.01. The omitted element is: the *Pichia* strain must also be transformed with a nucleotide sequence coding for a heterologous glycoprotein so that the transformed cells of said *Pichia* strain can produce the heterologous glycoprotein. As written, there is no linkage between the preamble of the claims reciting "reducing glycosylation of a heterologous glycoprotein" with the body of the claim simply reciting transforming cells of a *Pichia* strain with a nucleotide sequence coding for a *T. reesei* α-1,2-mannosidase or its functional part thereof and a nucleotide

sequence comprising a portion of the genomic OCH1 gene of said strain operably linked to a selectable marker.

Additionally, independent claim 107 recites the limitation "said functional part thereof" in lines 3 and 5 of the claim. There is insufficient antecedent basis for this limitation in the claim. This is because prior to these limitations, there is no recitation of functional part of anything. Accordingly, the metes and bounds of the claims are not clearly determined.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

New claims 90-96 and 104-113 are rejected under 35 U.S.C. 102(e) as being anticipated by Gerngross (US 2002/0137134; IDS) as evidenced by JP 8-336387 (IDS). ***This is a modified rejection necessitated by Applicant's amendment.***

Gerngross discloses methods and compositions by which fungi or other eukaryotic microorganisms including *Pichia pastoris*, *Hansenula polymorpha, Candida albicans* can be genetically modified to produce glycosylated proteins having patterns of glycosylation similar to glycoproteins produced by animal cells, particularly human cells,

which are useful as human or animal therapeutic agents such as erythropoietin, cytokines, coagulation factors (See Summary of Invention, pages 4-5; page 6, paragraph 56). This is achieved using a combination of engineering and/or selection of **strains which do not express a certain enzymes that which create the undesirable complex structures characteristic of the fungal glycoproteins, which express exogenous enzymes selected either to have optimal activity under the conditions present in the fungi where activity is desired or which are targeted to an organelle where optimal activity is achieved** (including α-1,2-mannosidase from *Trichoderma reesei*, paragraph 68-71), **and combinations thereof** wherein the genetically engineered eukaryote expresses multiple exogenous enzymes required to produce "human-like" glycoproteins (see abstract; paragraphs 41, 48). Specifically, Gerngross teaches that **in a preferred process or embodiment** the microorganism is engineered to express an exogenous α-1,2-mannosidase enzyme having an optimal pH between 5.1 and 8.0, and that the enzyme is targeted the endoplasmic reticulum (ER) or Golgi apparatus of the host organism, where it trims N-glycans such as $Man_8GlcNAc_2$ to yield $Man_5GlcNAc_2$ which is a substrate for further glycosylation reactions that produce a finished N-glycan that is similar or identical to that formed in mammals and it is not a substrate for hypermannosylation reactions that occur *in vivo* in yeast or other microorganisms (paragraphs 42, 68-72; Table 6 and claim 1). Gerngross also teaches that ER or Golgi apparatus targeting sequences are well known in the art such as HDEL or KDEL (paragraphs 87-88 and Table 6); and where the host is *Pichia pastoris* suitable promoters such as AOX1, AOX2, DAS and P40 promoters can be used at least for

expression of an exogenous gene encoding glycosylation enzymes (paragraph 85 and example 1). Gerngross specifically teaches that the eukaryotic strains which do not express one or more enzymes involved in the production of high mannose structures are used, and that these strains can be engineered in conjunction with the introduction of an exogenous α-1,2-mannosidase enzyme (paragraphs 64-67), and one of the many such mutants already described in yeasts including a hypermannosylation-minus (OCH1) mutant in *Pichia pastoris* described in Japanese Patent Application Public No. 8-336387 (paragraphs 35, 48). JP 8-336387 already teaches the preparation of a vector construct comprising a portion of *Pichia* OCH1 gene and a selectable marker gene for disruption of the genomic OCH1 in a *Pichia* yeast strain, including the GTS 115 (NRRL Y-15851) strain for inhibiting the elongation of sugar chains on glycoproteins (see abstract in English, Fig. 9 on page 635 as well as col. 15, paragraph 0033). It is further noted that articles (e.g., a cultured flask or a vial) containing vectors for expression of exogenous α-1,2-mannosidase enzyme and for disruption of the genomic OCH1 in a *Pichia* yeast strain and/or the eukaryotic microorgainisms (e.g., *Pichia* yeasts) in the invention of Gerngross would constitute such a kit.

Accordingly, the teachings of Gerngross meet every limitation of the instant claims. Therefore, the reference anticipates the instant claims.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on 5/3/07 (pages 15-17) have been fully considered but they are respectfully not found persuasive for the reasons discussed below.

1. Applicants argue that Gerngross does not teach the strains and methods as presently claimed, particularly *Pichia pastoris* that is transformed with a vector capable of expressing a *T. reesei* α-1,2-mannosidase or a functional part thereof. Instead, Gerngross teaches away from such a strain because Gerngross discloses that to obtain $Man_5GlcNAc_2$ in high yield, one could engineer a strain that expresses a α-1,2-mannosidase which should have an optimal pH that is between 5.1 and 8.0, while the *T. reesei* α-1,2-mannosidase has an optimal pH of 5.0 and Gerngross discusses the inefficiency of this enzyme in several passages.

Firstly, it should be noted that although Gerngross discloses a genetically engineered microorganism expressing an exogenous α-1,2-mannosidase having an optimal pH between 5,1 and 8.0, however this is only a preferred process or a preferred embodiment of the teachings of Gerngross, particularly if one desires to have an **"efficient"** production of $Man_5GlcNac_2$ in vivo (see paragraphs 42, 68-72 and Table 6). The Gerngross reference does not teach explicitly that *T. reesei* α-1,2-mannosidase should not be used even though the reference acknowledges that this mannosidase as well as the *Aspergillus saitoi* α-1,2-mannosidase having a pH optimum around pH 5.0 are capable of producing $Man_5GlcNac_2$ *in vivo* even though they are not efficient (see at least paragraph 71). Moreover, Gerngross also teaches the use of exogenous enzymes selected either to have optimal activity under the conditions present in the fungi where

activity is desired **or which are targeted to an organelle where optimal activity is achieved**, such as a HDEL-*T. reesei* α-1,2-mannosidase fusion protein targeted to ER in a *Pichia pastoris* strain. This chimeric *T. reesei* α-1,2-mannosidase fusion protein would have intracellular enzymatic activity whereas the native *T. reesei* α-1,2-mannosidase does not have any intracellular enzymatic activity in a *Pichia pastoris* strain as evidenced at least by the teachings of Martinet et al. (Biotechnology Letters 20:1171-1177, 1998; IDS) discussed below.

Secondly, the rejected claims do not require the genetically engineered *Pichia* yeast strain to produce $Man_5GlcNAc_2$ to any degree of efficiency or any particular fraction of produced glycoproteins having the structure $Man_5GlcNAc_2$. Please also note that the term "reduced glycosylation" as defined by the instant specification to mean a reduced size of the carbohydrate moiety on the glycoprotein, particularly with fewer mannose residues, when the glycoproteinis expressed in a methylotrophic yeast strain which has been modified in accordance with the present invention, as compared to a wild type, unmodified strain of the methylotrophic yeast (page 28, lines 1-5).

2. With respect to the teaching of Gerngross relating to Och1, Gerngross merely discloses reducing endogenous mannosyltransferase activity and based on the disclosure of Gerngross it is unclear whether the disruption of OCH1 alone in a *Pichia* strain would be sufficient or whether multiple enzymes need to be disrupted in order to obtain Man_8 and ultimately Man_5 N-glycans. Although Gerngross refers to JP 8-336387 for an Och1 mutant strain of *P. pastoris*, this Japanese application does not appear to

provide any showing that the predominant N-glycan form in the mutant is a Man_8 glycan. Applicants further argue that a recent report by Choi et al (Exhibit 1) showed that Man_9 is still a predominant N-glycan form in the Och1 mutant (Figure 3B).. Accordingly, Gerngross merely provides numerous potential options for those skilled in the art to experiment, and does not provide clear teaching that anticipates the presently claimed invention.

Firstly, with respect to Applicants' argument on whether multiple enzymes need to be disrupted in order to obtain Man_8 and ultimately Man_5 N-glycans, it is irrelevant because the claims do not require the genetically engineered yeast *Pichia* strain only having the disruption of OCH1 alone, and due to the open language of the term "comprising" in method claims.

Secondly, Figure 3 B in the post-filing art of Choi et al (Exhibit 1) only showed the released N-linked glycans analysis from K3 produced in och1 deleted *P.pastoris* strain BK64-1, not from och1 deleted *P. pastoris* strain expressing an exogenous α-1,2-mannosidase. Furthermore, Choi et al stated "Fungal α-1,2-mannosidase with acidic pH optima (e.g., *P citrinium* and *A. nidulans*), when expressed as fusions with the leader library, generally **resulted in low $(Man)_5(GlcNAc)_2$ yields (data not shown) consistent with previous findings (11, 21)**" (page 5026, col. 1, bottom of second full paragraph).

Accordingly, new claims 90-96 and 104-113 are still rejected under 35 U.S.C. 102(e) as being anticipated by Gerngross (US 2002/0137134; IDS) as evidenced by JP 8-336387 (IDS) for the reasons set forth above.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

New claims 90-91, 93-96, 104-108, 110 and 112-113 are rejected under 35 U.S.C. 103(a) as being unpatentable over Martinet et al. (Biotechnology Letters 20:1171-1177, 1998; IDS) in view of JP 8336387 (12/24/96; IDS) as evidenced by Choi et al. (PNAS 100:5022-5027, 2003; Cited by Applicants). ***This is a modified rejection necessitated by Applicant's amendment.***

Martinet et al. teaches the preparation of plasmids for expression of *T. reesei* α-1,2-mannosidase or a chimeric *S. cerevisiae/ T. reesei* α-1,2-mannosidase (a fusion of

the catalytic domain of *T. reesei* α-1,2-mannosidase to the ER retention signal of *S. cerevisiae* MNS1) in *Pichia pastoris* strains GSIV-HAs and GSIVNAf1s derived from the parental strain GS115 (see Materials and Methods, particularly sections "Strains and culture conditions" and "Construction of plasmids for expression of *T. reesei* α-1,2-mannosidase in *P. patoris*"). Martinet et al. further teaches that in all expression plasmids are derived from the pPICZB vector, and the *T. reesei* α-1,2-mannosidase gene was under transcriptional control of the AOX1 promoter (page 1172, col. 2, first full paragraph). Martinet et al. also discloses that co-expression of heterologous *T. reesei* α-1,2-mannosidase in GSIVNAf1s resulting in partial trimming of the large influenza neuramidase (NA) N-glycans (>Man14GlcNac2) (see section "In vivo trimming of N-glycans by heterologous *T. reesei* α-1,2-mannosidase", and Figures 2A, 3). The co-expression of the chimeric MNS1/*T. reesei* α-1,2-mannosidase in GSIV-HAs resulted in the formation of both trimmed and hyperglycosylation glycan products of hemagglutinin (HA) (see page 1175, col. 2 and Fig. 4). Additionally, Martinet et al. notes that hyperglycosylation can be prevented by expression the protein of interest in the mutant yeast strains *mnn9, och1* or in the temperature-sensitive strain *ngd-29*, where N-glycosylation is confined to the core oligosaccharide residues (page 1176, col. 1); and the results from the co-expression of the chimeric MNS1/*T. reesei* α-1,2-mannosidase in GSIV-Has suggest that removal of mannose residues creates more ideal substrates for P. pastoris mannosyltransferrases, leading to elongation of truncated glycosyl chains, and not to complete α-1,2-mannosidase digestion (page 1175, bottom of col. 2).

Martinet et al. does not teach to further transform the *Pichia pastoris* strain with a vector comprising a portion of the Och1 gene and a selectable marker gene to effect the disruption of the genomic Och1 gene in the *Pichia pastoris* strain to reduce the glycosylation of a heterologous glycoprotein or producing a glycoprotein with reduced glycosylation; and a kit comprising the vectors and/or these further genetically modified *Pichia pastoris* yeasts.

However, at the effective filing date of the present application, JP 8-336387 already taught the preparation of a vector construct comprising a portion of *Pichia* OCH1 gene and a selectable marker gene for disruption of the genomic OCH1 in a *Pichia* yeast strain, including the GTS 115 (NRRL Y-15851) strain for inhibiting the elongation of sugar chains on glycoproteins for production of a glycoprotein having a sugar chain identical or similar to that of a medically useful biologically active protein (see at least the abstract in English, Fig. 9 on page 635 as well as col. 15, paragraph 33).

Accordingly, it would have been obvious and within the scope of skill for an ordinary artisan to modify the method and compositions taught by Martinet et al. by at least further transforming *Pichia pastoris* strains GSIV-HAs and GSIVNAf1s expressing heterologous *T. reesei* α-1,2-mannosidase using a vector construct comprising a portion of the *Pichia* OCH1 gene and a selectable marker gene for disruption of the genomic OCH1 taught by JP 8336387.

An ordinary skilled artisan would have been motivated to carry out the above modification because the elimination of endogenous OCH1 in a *Pichia* yeast strain

inhibits the addition of α-1,6-polymannose outer chain formation on the Asn-linked inner core oligosaccharide Man8GlcNAc2, and results in smaller and homogenous oligosaccharides in heterologous glycoproteins or at least production of a glycoprotein having a sugar chain identical or similar to that of a medically useful biologically active protein as taught by JP 8-336387. Moreover, Martinet et al. already noted at least that hyperglycosylation can be prevented by expression the protein of interest in the mutant yeast strains *mnn9, och1* or in the temperature-sensitive strain *ngd-29*, where N-glycosylation is confined to the core oligosaccharide residues (page 1176, col. 1). The kits comprising the vector components for carrying out the modified methods and genetically modified *Pichia pastoris* yeast strains discussed above would also have been obvious. Furthermore, the genetically modified *Pichia pastoris* yeast strains resulting from the combined teachings of Martinet et al. and JP 8-336387 are capable of producing $Man_5GlcNAc_2$ as evidenced at least by the teachings of Choi et al which disclose fungal α-1,2-mannosidase with acidic pH optima (e.g., *P citrinium* and *A. nidulans*), when expressed as fusions with the leader library, in a *P. pastoris* och1 mutant strain generally resulted in low $(Man)_5(GlcNAc)_2$ yields consistent with previous findings (page 5026, col. 1, second full paragraph).

An ordinary skilled artisan would have a reasonable expectation of success to carry out the above modification in light of the teachings of Martinet et al., and JP 8-336387, coupled with a high level of skills of an ordinary skilled artisan in the relevant art.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on 5/3/07 (pages 17-18) have been fully considered but they are respectfully not found persuasive for the reasons discussed below.

Applicants argue basically that Martinet et al reference refers to the *S. cerevisiae* mutant strains for preventing hyperglycosylation as evidenced by Exhibit 2, and that the reference also notes that the glycosylation pathways of *S. cerevisiae* and *P. pastoris* are significantly different. Applicants further argue that Man_9 is still a predominant N-glycan form in the Och1 mutant of *Pichia pastoris* as taught by Choi et al (Exhibit 1); and a skilled artisan would have expected to produce the Man_5 glycan structure from a Man_8 glycan, not from Man_9. Therefore, it would not have been obvious for an ordinary skilled artisan based on the disclosure of Martinet et al and JP 8336387 to obtain a *Pichia pastoris* strain as presently claimed and that is capable of producing the desired Man_5 glycan structure.

Firstly, it appears that Applicants considered each of the cited references in total isolation one from the other. JP 8-336387 already taught the preparation of a vector construct comprising a portion of *Pichia* OCH1 gene and a selectable marker gene for disruption of the genomic OCH1 in a *Pichia* yeast strain, including the GTS 115 (NRRL Y-15851) strain for inhibiting the elongation of sugar chains on glycoproteins for

production of a glycoprotein having a sugar chain identical or similar to that of a medically useful biologically active protein. Particularly, the primary Martinet et al reference already notes that hyperglycosylation can be prevented by expression the protein of interest in the mutant yeast strains *mnn9, och1* or in the temperature-sensitive strain *ngd-29*, where N-glycosylation is confined to the core oligosaccharide residues (page 1176, col. 1); and the results from the co-expression of the chimeric MNS1/*T. reesei* α-1,2-mannosidase in GSIV-Has suggest that removal of mannose residues creates more ideal substrates for *P. pastoris* mannosyltransferrases, leading to elongation of truncated glycosyl chains, and not to complete α-1,2-mannosidase digestion (page 1175, bottom of col. 2). Although the glycosylation pathways of *S. cerevisiae* and *P. pastoris* are significantly different, inactivated mutations including deletion of endogenous Och1 in either *S. cerevisiae* or *P. pastoris* strain would prevent or reduce hyperglycosylation.

Secondly, Figure 3 B in the post-filing art of Choi et al (Exhibit 1) only showed the released N-linked glycans analysis from K3 produced in och1 deleted *P.pastoris* strain BK64-1, not from och1 deleted *P. pastoris* strain expressing an exogenous α-1,2-mannosidase. Furthermore, Choi et al stated "Fungal α-1,2-mannosidase with acidic pH optima (e.g., *P citrinium* and *A. nidulans*), when expressed as fusions with the leader library, generally **resulted in low $(Man)_5(GlcNAc)_2$ yields (data not shown) consistent with previous findings (11, 21)**" (page 5026, col. 1, bottom of second full paragraph).

Thirdly, the rejected claims do not require the genetically engineered *Pichia* yeast strain to produce $Man_5GlcNAc_2$ to any degree of efficiency or any particular fraction of produced glycoproteins having the structure $Man_5GlcNAc_2$. Please also note that the term "reduced glycosylation" as defined by the instant specification to mean a reduced size of the carbohydrate moiety on the glycoprotein, particularly with fewer mannose residues, when the glycoproteinis expressed in a methylotrophic yeast strain which has been modified in accordance with the present invention, as compared to a wild type, unmodified strain of the methylotrophic yeast (page 28, lines 1-5).

Accordingly, new claims 90-91, 93-96, 104-108 and110-113 are rejected under 35 U.S.C. 103(a) as being unpatentable over Martinet et al. (Biotechnology Letters 20:1171-1177, 1998; IDS) in view of JP 8336387 (12/24/96; IDS) as evidenced by Choi et al. (PNAS 100:5022-5027, 2003; Cited by Applicants) for the reasons set forth above.

In light of the Supreme Court Decision in KSR International Co. v. Teleflex Inc., 550 U.S.—82 USPQ2d 1385 (2007), the following rejection is applied.

New claims 97-106 are rejected under 35 U.S.C. 103(a) as being unpatentable over Martinet et al. (Biotechnology Letters 20:1171-1177, 1998; IDS) in view of JP 8336387 (12/24/96; IDS) as evidenced by Choi et al. (PNAS 100:5022-5027, 2003; Cited by Applicants) as applied to claims 90-91, 93-96, 104-108, 110 and 112-113 above, and further in view of Trombetta et al. (J. Biol. Chem. 271:27509-27516, 1996;

IDS) and Chiba et al. (J. Biol. Chem. 273:26295-26304, 1998; IDS). ***This is a new ground of rejection necessitated by Applicant's amendment.***

The combined teachings of Martinet et al and JP 8336387 were already discussed above. However, none of the cited references teaches specifically that the genetically modified yeast *Pichia* further transformed with a vector comprising a nucleotide sequence coding for a glucosidase II or a functional part thereof or the use of a GAP promoter for expressing either *T. reesei* α-1,2-mannosidase and/or glucosidase II.

However, at the effective filing date of the present application Trombetta et al. already disclosed cDNA sequences encoding endoplasmic reticulum glucosidase II derived from various sources; including a glucosidase II gene from *S.cerevisiae* (see at least the abstract and sections titled "Primary sequence of αSubunit" and "Identification of the *S. cerevisiae* functional homologue of mammalian glucosidase II catalytic subunit (α)" on pages 27511-27513). Trombetta et al further demonstrated that *S.cerevisiae* functional homologue of mammalian glucosidase II catalytic subunit α removes two α-1,3-linked Glc units after removal of the terminal α-1,2-linked Glc residue in the core oligosaccharide $Glc_3Man_9GlcNAc2$ (page 17514, col. 1, second paragraph).

Additionally, Chiba et al already taught the preparation of an expression vector encoding HDEL-tagged *Aspergillus* α-1,2-mannosidase for expression in various *Saccharomyces cerevisiae* strains, named pGAMH1 plasmid containing the GAP promoter (see abstract and the section "DNA constructs. Chiba et al. further taught that carboxypeptidase Y produced in the YS132-8B yeasts having disrupted OCH1, MNN1

and MNN4 genes and harboring pGAMH1 plasmid has trimmed sugar chains up to $Man_5GlcNAc_2$, instead of carboxypeptidase Y containing high mannose type sugar chains in wild type *Saccharomyces cerevisiae* (see Fig. 1, and page 26302, col. 1, first full paragraph).

Accordingly, it would have been obvious and within the scope of skill for an ordinary artisan to further modify the combined teachings of Marinet et al. and JP 8336387 by further transforming a modified och-1 mutant *Pichia pastoris* strain expressing an exogenous *T. reesei* α-1,2-mannosidase tagged with an ER-retention signal with a recombinant vector expressing an exogenous ER glucosidase II, as well as the use of the GAP promoter for the expression of either the exogenous glucosidase II or *T. reesei* α-1,2-mannosidase in light of the teachings of Trombetta et al and Chiba et al as discussed above.

An ordinary skilled artisan would have been motivated to carry out the above modifications because expressing an exogenous glucosidase II in the modified och-1 mutant *Pichia pastoris* strain expressing heterologous *T. reesei* α-1,2-mannosidase would enhance the removal of any unprocessing glucose residues, and thus enhancing the enzymatic activity of the heterologous *T. reesei* I-1,2-mannosidase to result in a more complete trimming of sugar chains in heterologous glycoproteins expressing in these further modified *Pichia* yeast strains. Particularly, Martinet et al. already suggested that **unprocessed glucose residues or capping glucose residues may block α-1,2-mannosidase treatment, and that these may be responsible for incomplete trimming of NA oligosaccharides to Man5GlcNAc2** (page 1175, col. 2,

first paragraph; page 1176, col.1, bottom of the second paragraph). Additionally, GAP promoter has been successfully used for expressing HDEL-tagged *Aspergillus* α-1,2-mannosidase in yeasts as already demonstrated by Chiba et al for generating heterologous carboxypeptidase Y having trimmed sugar chains up to $Man_5GlcNAc_2$.

An ordinary skilled artisan would have a reasonable expectation of success to carry out the above modifications in light of the teachings of Martinet et al., JP 8-336387, Trombetta et al. and Chiba et al., coupled with a high level of skills of an ordinary skilled artisan in the relevant art.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970);and, *In re Thorington,* 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

New claims 90-96 and 105 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claim 9 of U.S. Patent No. 7,252,933. ***This is a new ground of rejection necessitated by Applicant's amendment.***

Although the conflicting claims are not identical, they are not patentably distinct from each other because a methylotrophic yeast strain of any one of claims 1, 2, 3, 4-5 or 6-7, wherein the expression of said *T.reseei* α-1,2-mannosidase in said strain is directed by a promoter, wherein said promoter is the promoter of a gene selected from the group consisting of AOXI, an AOXII, GAP, YPT1 and FLD in the issued US Patent 7,252,933 anticipates the claimed genus of a genetically engineered strain of *Pichia* and a kit comprising the same in the application being examined and, therefore, a patent to the genus would, necessarily, extend the rights of the species or sub- should the genus issue as a patent after the species of sub-genus.

New claims 90-96 and 105 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 39, 41-59 of copending Application No. 10/713,970. ***This is a new ground of rejection necessitated by Applicant's amendment.***

Although the conflicting claims are not identical, they are not patentably distinct from each other because a genetically engineered *Pichia* strain, wherein said strain is engineered to express (1) a *Trischoderma reesei* α-1,2-mannosidase or a functional part thereof, (2) an N-acetylglucosaminyltransferase I (GnTI) or a functional part thereof,

and (3) a beta-1,4-galactosyltransferase (GalT) or a functional part thereof, and the genomic OCH1 gene of said strain is disrupted in the copending Application No. 10/713,970 anticipates the claimed genus of a genetically engineered strain of *Pichia* and a kit comprising the same in the application being examined and, therefore, a patent to the genus would, necessarily, extend the rights of the species or sub- should the genus issue as a patent after the species of sub-genus.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

New claims 90-113 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 5-8, 13-14 and 17-28 of U.S. Patent No. 6,803,225. ***This is a new ground of rejection necessitated by Applicant's amendment.***

The claims of the present application differ from the claim of the issued US Patent 6,803,225 in reciting specifically a genetically engineered Pichia yeast strain expressing *T. reesei* α-1,2-mannosidase, and a recited Markush group of specific promoters used to express *T. reesei* α-1,2-mannosidase and/or glucosidase II.

The claims of the present application can not be considered to be patentably distinct over claims 5-8, 13-14 and 17-28 of U.S. Patent No. 6,803,225 when there is a specific disclosed embodiment of the issued US patent that teaches the use of vectors coding for *T. reesei* α-1,2-mannosidase and its expression under the control of at least

AOX1 promoter (see all the examples). Accordingly, the claims of the issued US patent fall within the scope of claims 90-113 of the present application.

This is because it would have been obvious to an ordinary skilled artisan to modify the claims of the issued US patent by also using vectors coding for *T. reesei* α-1,2-mannosidase and its expression under the control of at least AOX1 promoter for making and using the genetically engineered *Pichia* yeast strain, that support the instant claims. An ordinary skilled artisan would have been motivated to do this because this embodiment is explicitly disclosed or taught in the issued US patent as a preferred embodiment.

Conclusion

No claim is allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of

the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Quang Nguyen, Ph.D., whose telephone number is (571) 272-0776.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's SPE, Joseph T. Woitach, Ph.D., may be reached at (571) 272-0739.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1633; Central Fax No. (571) 273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

Patent applicants with problems or questions regarding electronic images that can be viewed in the Patent Application Information Retrieval system (PAIR) can now contact the USPTO's Patent Electronic Business Center (Patent EBC) for assistance. Representatives are available to answer your questions daily from 6 am to midnight (EST). The toll free number is (866) 217-9197. When calling please have your application serial or patent number, the type of document you are having an image problem with, the number of pages and the specific nature of the problem. The Patent Electronic Business Center will notify applicants of the resolution of the problem within 5-7 business days. Applicants can also check PAIR to confirm that the problem has been corrected. The USPTO's Patent Electronic Business Center is a complete service center supporting all patent business on the Internet. The USPTO's PAIR system provides Internet-based access to patent application status and history information. It also enables applicants to view the scanned images of their own application file folder(s) as well as general patent information available to the public.

/QUANG NGUYEN, Ph.D./

Primary Examiner, Art Unit 1633